Amendments to the Specification

Please amend Example 1 of the specification starting at page 5, line 4 as follows:

The formulations shown in Table I were made.

Table 1

Sample	[AAT] mg/ml	pH	NaPi	Histidine	NaCl	Citrate	NAC	L-m
917-1	50	7	20	0	175	5	2.5	3
917-3	50	7	20	0	100	5	2.5	3
917-4	50	7	20	0	50	5	2.5	3
917-11	50	7	20	0	0	0	0	0

In order to assess the conformational stability of rAAT in the dried state, FTIR spectra were collected on these formulations. It has been shown that retention of native structure in the solid state can be predictive of long-term storage stability for dried proteins (Carpenter et al, 2002, supra). Figure 1 shows the FTIR of liquid and solid rAAT in Formulation 917-1. Note that the amide I region (1700-1600 cm^{-1}) is sensitive to changes in secondary structure and that all peaks in the second derivative spectra are negative. Each peak in the amide I region corresponds to a different secondary structural type. There are clearly perturbations of the rAAT conformation before and after lyophilization. The peak near 1655 cm.sup.-1 corresponds to α-helical structure, the band near 1635 cm^{-1} corresponds to β-sheet structure, and the 1688 cm.sup.-1 peak arises from extended β-strands or β-sheets. Random coil structure is assigned to bands near 1644 cm^{-1}.

The liquid sample, representing the native conformation, displays a significant amount of β-sheet and α-helical structure. Upon lyophilization, without stabilizers (formulation 917-11), there is significant structural perturbation as shown in Figure 2. The α-helix band is almost completely lost, while there are marked increases in bands above 1680 cm^{-1}, corresponding to

extended and loop structures. Figure 3 shows the effect of salt on rAAT structure in the solid state. Formulations 917-1, -3 and -4 contain 175 mM, 100 mM and 50 mM NaCl, respectively, in addition to 20 mM sodium phosphate, 5 mM citrate, 2.5 mM NAC, and 3 mM L-Met.

Formulations 3 and 4, which have the lower salt concentrations, appear to have the greatest degree of structural perturbation and all three formulations are less perturbed than when no stabilizers are present. Overall, it appears that lyophilization produces some structural perturbation compared to the native conformation. The extent of the changes is minimized by the addition of excipients, including salt. It appears that a NaCl concentration above 50 mM produces a more native-like structure, with a 50-100 mM optimum. The result is unanticipated, since sugars are usually required or used to maintain native protein structure in the dried state. Conformational stability of these formulations was also assessed by FTIR in order to elucidate any subtle differences between the rAAT structure in the dried state. Figure 4 shows the FTIR spectra of rAAT formulated in a sugar-based formulation (1008-1) and in a salt-based formulation (1008-2). The secondary structure of rAAT in both these formulations is superimposable. The fact that salt can accomplish the same degree of stabilization with protein at high concentrations is remarkable and not obvious. Upon reconstitution, the original rAAT secondary structure is retained as shown in Figure 5.

Based on the surprising observations of lyophilized rAAT formulations containing high levels of NaCl, stability analysis is ~~were~~ done in order to evaluate systematically whether addition of common stabilizers in lyophilized protein formulations enhances the conformational stability and acute stability (3 month storage at 60°C.) of rAAT. Sugars are commonly used in protein formulations to stabilise the molecule by presumably substituting for the H-bonding following removal of the water molecules around the protein during lyophilization. Sugars also offer an amorphous environment in the dry state that promotes conformational stability of the protein, and they effectively replace the water of hydration removed during drying. Surfactants are also often employed in protein formulations to reduce surface adsorption that may damage the protein. Since a possible administration route for rAAT is pulmonary delivery via aerosolization, the effect of surfactant is especially of interest. Therefore, the role of

polyoxyethylene sorbitan, such as polysorbate 80 (Tween 80), at various concentrations is ~~was~~ also evaluated. These formulations are given in Table II.

Table II

Sample	pH	NaPi	Trehalose	Sucrose	Tw80	NaCl	L-met	NAC	Citrate
		mM	%	%	%	mM	mM	mM	mM
1008-1	7.4	10	5	0	0	100	5	0	0
1008-2	6.8	10	0	0	0	100	3	0	0
1008-3	6.8	10	0	0	0	100	3	0	0
1008-4	6.8	10	2.5	0	0	100	3	0	0
1008-5	6.8	10	2.5	0	0	100	3	0	0
1008-6	6.8	10	2.5	0	0	100	3	0	0
1008-7	6.8	10	0	2.5	0	100	3	0	0
1008-8	7.4	10	0	0	0	100	3	0	0
1008-9	6.8	10	0	0	0	100	3	2.5	5
1008-10	6.8	10	0	1	0	100	3	0	0
1008-11	6.8	10	0	2.5	0	100	3	0	0
1008-12	6.8	10	0	5	0	100	3	0	0

The lyophilized formulations are ~~were~~ evaluated for short-term stability (at 1 and 3 months) under accelerated storage conditions at 60°C. It should be noted that this storage temperature is particularly harsh for evaluating protein stability and may bias the results towards the trehalose-based formulations that have a particularly high glass-transition temperature (Tg). The rationale for choosing this temperature is ~~was~~ based on previous stability studies that assessed rAAT stability over shorter time frames. The activity and percent monomer recovered

are ~~were~~ determined for up to 3 months storage at 60°C~~, as shown in Tables III and IV, respectively~~.

~~Table III: Specific Activity of rAAT (IU/mg)~~

~~Sample~~	~~Pre-lyo~~	~~Lyo (1 month RT)~~	~~Moisture~~	~~Lyo (1 month 60°C)~~	~~Lyo (3 month 60°C)~~
~~Liquid control~~	~~3.75~~				
~~1008-1~~	~~3.6~~	~~3.45~~	~~0.4 %~~	~~3.42~~	~~2.4~~
~~1008-2~~	~~3.69~~	~~2.83~~	~~1.4 %~~	~~2.89~~	~~2~~
~~1008-3~~	~~4.02~~	~~3.22~~		~~3.21~~	~~2~~
~~1008-4~~	~~3.57~~	~~3.47~~	~~0.6 %~~	~~3.31~~	~~2.6~~
~~1008-5~~	~~3.67~~	~~3.21~~		~~3.24~~	~~2.7~~
~~1008-6~~	~~3.7~~	~~3.10~~		~~3.43~~	~~2.5~~
~~1008-7~~	~~3.89~~	~~3.08~~		~~3.22~~	~~2.6~~
~~1008-8~~	~~3.43~~	~~3.15~~		~~3.09~~	~~2.3~~
~~1008-9~~	~~3.57~~	~~3.16~~		~~3.23~~	~~2.5~~
~~1008-10~~	~~3.38~~	~~3.16~~		~~3.12~~	~~2.7~~
~~1008-11~~	~~4.04~~	~~3.28~~	~~0.4 %~~	~~3.2~~	~~2.7~~
~~1008-12~~	~~3.31~~	~~3.31~~		~~3.18~~	~~3~~

~~Table IV: Percent Monomer by Size Exclusion HPLC~~

~~Sample~~	~~Pre-lyo~~	~~Lyo (1 month RT)~~	~~Lyo (1 month 60°C)~~	~~Lyo (3 month 60°C)~~
~~Liquid control~~	~~97.6~~			
~~1008-1~~	~~98.2~~	~~97.02~~	~~96.7~~	~~74.71~~
~~1008-2~~	~~97.4~~	~~95.65~~	~~96.7~~	~~65.57~~
~~1008-3~~	~~97.4~~	~~96.03~~	~~94.89~~	~~60.62~~
~~1008-4~~	~~97.4~~	~~96.04~~	~~96.3~~	~~85.85~~
~~1008-5~~	~~97.4~~	~~96.36~~	~~96.07~~	~~86.94~~
~~1008-6~~	~~97.3~~	~~96.73~~	~~96.16~~	~~83.2~~
~~1008-7~~	~~97.4~~	~~96.33~~	~~95.29~~	~~86.76~~
~~1008-8~~	~~97.5~~	~~96.3~~	~~94.26~~	~~65.46~~
~~1008-9~~	~~97.1~~	~~96.03~~	~~93.12~~	~~74.32~~
~~1008-10~~	~~97.2~~	~~96.3~~	~~94.35~~	~~84.29~~
~~1008-11~~	~~97.3~~	~~95.72~~	~~95.03~~	~~82.83~~
~~1008-12~~	~~97.2~~	~~96.19~~	~~95.96~~	~~5.41~~

~~No significant differences were observed in any of the formulations tested after 1 month, suggesting that both sugar-containing and sugar-free formulations offer comparable stability.~~

~~The stability data after storage for 3 months at 60°C display more variable results. It appears that formulations containing both sugar and salt have a better stability profile than those containing either sugar or salt alone. These data are consistent with FTIR studies that show a high degree of retention of secondary structure in these types of formulations. The low specific activity seen in formulation 1008-2 may be due to the moisture content in that formulation, which is almost 1% higher than that determined in the other selected formulations. This suggests that stable lyophilized rAAT formulations should preferably have a moisture content below 1%. These results suggest that rAAT is a relatively stable protein and may not require sugars for stabilization in the lyophilized state.~~